J. Physiol. (1980), **302**, *pp.* 549–557
With 4 text-figures
Printed in Great Britain

VASOACTIVE INTESTINAL POLYPEPTIDE, 5-HYDROXYTRYPTAMINE AND REFLEX HYPERAEMIA IN THE SMALL INTESTINE OF THE CAT

BY S. EKLUND*, J. FAHRENKRUG, M. JODAL*, O. LUNDGREN*, O. B. SCHAFFALITZKY DE MUCKADELL AND A. SJÖQVIST*

From the Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark and the Department of Physiology, University of Göteborg, Sweden*

(*Received 17 May 1979*)

SUMMARY

1. The release of vasoactive intestinal polypeptide (VIP) into venous blood from the small intestine of the cat was studied when mechanically stimulating the intestinal mucosa and during close intra-arterial infusions of 5-hydroxytryptamine (5-HT) or isopropylnoradrenaline. The studies were performed on anaesthetized cats given atropine.

2. Mechanical stimulation of the intestinal mucosa induced a vasodilatation and a release of VIP into the intestinal venous blood. Intra-arterial administration of tetrodotoxin was given in doses that blocked the vasoconstrictor effect of the regional sympathetic nerve fibres. This also abolished the vascular response and the release of VIP into blood upon mechanical stimulation.

3. Close intra-arterial administration of 2-bromo-lysergic acid diethylamide reduced the VIP release and the intestinal vasodilatation upon mucosal stimulation to largely the same extent.

4. Close intra-arterial infusions of 5-HT produced a marked release of VIP from the intestine and a moderate vasodilatation. Close intra-arterial infusions of isopropylnoradrenaline, which caused a pronounced intestinal vasodilatation, evoked only a small release of VIP.

5. The results are compatible with the hypothesis that the vasodilatation in the gut, induced by mechanical mucosal stimulation, is mediated via an intramural nervous reflex containing a neurone capable of releasing VIP. It is proposed that the nervous reflex is activated by the release of 5-HT from the enterochromaffin cells evoked by mechanical stimulation of the mucosa.

INTRODUCTION

Mechanical stimulation of the intestinal mucosa elicits a marked hyperaemia in the feline gut (Biber, Jodal, Lundgren & Svanvik, 1970; Biber, Lundgren & Svanvik, 1971). This vasodilatation is evidently induced by the activation of a local intramural nervous reflex since it is blocked by intraluminal administration of lidocaine, a local anaesthetic agent, or by intra-arterial administration of tetrodotoxin, a nerve

0022-3751/80/8260-0222 $07.50

conduction blocking agent (Biber *et al.* 1971). Furthermore, a pharmacological analysis (Biber *et al.* 1971; Biber, Fara & Lundgren, 1974) revealed that this nervous reflex hyperaemia was not blocked by the conventional adrenergic, cholinergic or ganglionic receptor blocking agents. On the other hand, blocking the 5-hydroxytryptamine (5-HT) receptors in the small intestine abolished the vascular response on mechanical intestinal stimulation. Vasoactive intestinal polypeptide (VIP), a basic octacosapeptide with potent vasodilator activity, was recently demonstrated

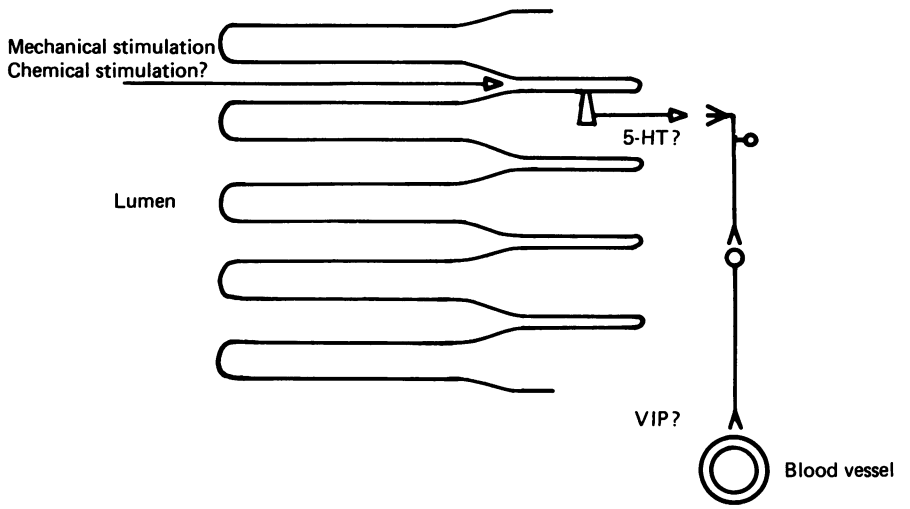

Fig. 1. Hypothetical arrangement of the nervous reflex underlying the vasodilator response seen upon mechanical stimulation of the intestinal mucosa. It is proposed that the mechanical stimulation releases 5-hydroxytryptamine (5-HT) from the enterochromaffin cells which, in turn, activate a nervous reflex arc which contains a neurone releasing vasoactive intestinal polypeptide (VIP) as a transmitter. The number of neurones in the arc is arbitrarily set at two but the existence of a synapse has not been shown. For details see text.

to be released from the intestine upon mechanical stimulation of the mucosa (Fahrenkrug, Haglund, Jodal, Lundgren, Olbe & Schaffalitzky de Muckadell, 1978). VIP has an unbiquitous occurrence in the body localized in neurones (Larsson, Fahrenkrug, Schaffalitzky de Muckadell, Sundler, Håkansson & Rehfeld, 1976). The VIP-containing nerves are particularly frequent in the gut and seem to innervate small vessels and smooth muscle cells.

From the observations briefly summarized above the hypothetical reflex arrangement illustrated in Fig. 1 may be envisaged. (For a more detailed discussion of this reflex arrangement see Biber *et al.* (1974) and Fahrenkrug *et al.* (1978)). According to Fig. 1 the mechanical and/or chemical stimulation of the enterochromaffin cells release 5-HT, stimulating free nerve endings located adjacent to the cells (Biber, 1973), which, in turn, activate the reflex arc. The number of neurones involved in the reflex is arbitrarily set to two in Fig. 1 but the presence of a nervous synapse has not been demonstrated.

The hypothetical reflex arrangement of Fig. 1 forms the basis for the present experiments. According to Fig. 1 it should be possible to diminish or abolish the

release of the VIP, observed upon mechanical stimulation of the mucosa, by administering a nerve conductivity blocking agent or a 5-HT receptor blocking agent. Furthermore, the intravascular administration of 5-HT should release VIP from the gut. The results of such experiments are reported below.

METHODS

Operative procedures

Experiments were performed on sixteen cats of both sexes weighing 2·0–4·5 kg. The animals were deprived of food for 24 hr with free access to water. Anaesthesia was induced with ether and maintained with chloralose I.V. (50 mg/kg body wt.). Body temperature was kept around 38 °C by means of a heating pad under the animal.

The abdomen was opened in the mid line after insertion of a tracheal cannula and one or two jejunal segments below flexura duodeno-jejunalis were chosen for the experiment. The length of the segment was about 15 cm and the total weight including the mesenteric lymph nodes amounted to 20–35 g. The remainder of the small intestine, the spleen, the great omentum and the colon were extirpated. The splanchnic nerves were cut bilaterally. One adrenal gland was denervated and the other excluded from the circulation by ligatures.

After heparinizing the animal, the left femoral artery was cannulated and connected to a pressure transducer (Statham P23 AC) to record mean arterial pressure. After cannulation of the superior mesenteric vein the venous outflow from the jejunal segment was measured by an optical drop recorder unit operating an ordinate writer. The blood was returned to the animal via a funnel connected to a catheter in a jugular vein. During the experiments the jejunal segment was wrapped in saline-soaked gauze and kept at body temperature by means of a thermocouple recorder and a lamp. Recordings of arterial pressure and blood flow were made on a Grass polygraph.

In some experiments the distal ends of the severed splanchnic nerves were mounted on silver-ring electrodes. Square-wave stimuli were delivered from a Grass stimulator (S5) to activate the regional vasoconstrictor fibres. Stimulation characteristics were set at 5–8 V, 5 msec and 8 Hz.

Close I.A. injections into the superior mesenteric artery were made via a cannula retrogradely inserted into the arterial branch that supplies proximal parts of the colon.

Experimental procedures

In some experiments mechanical stimulation of the intestinal mucosa was performed by pulling a short piece of plastic tube gently back and forth through the jejunal lumen by means of soft strings tied at the ends of the tube. This stimulation procedure was repeated every 20 sec during a 2 min period.

The blood samples for VIP determinations were collected via a T-tube connected to the venous drop recording unit. The blood was collected in ice-cooled plastic tubes (volume 3 ml.) containing 100 μl. (1000 K.I.E.) of the protease inhibitor aprotinin (Trasylol®, Bayer AB, Leverkusen, G.F.R.). Venous samples were taken 10, 5 and 0 min before and 0·5, 1, 2, 10, 15 and 30 min after the start of the mechanical stimulation. The tubes were kept on ice until centrifugation. One ml. plasma was pipetted off and frozen at -20 °C.

In other experiments 5-HT or isopropylnoradrenaline were infused for 5 min through the arterial cannula in the superior mesenteric artery. Venous samples were then taken 10, 5, 0 min before and 0·5, 2, 4, 10, 15 and 30 min after the start of the infusion. The blood samples were collected and treated as described above.

In most experiments arterial samples were collected at regular intervals throughout the experiments.

Laboratory analysis

The concentration of VIP in plasma was measured radioimmunochemically. Details on accuracy, precision, sensitivity and specificity have been described previously (Fahrenkrug & Schaffalitzky de Muckadell, 1977, 1978). 5-HT in concentrations up to 0·5 g/l., concentrations which are far above those obtained during infusion, did not interfere in the VIP assay.

Calculations and statistical analysis

The rate of release of VIP from intestine into blood was calculated from the arterio–venous plasma VIP concentration difference, blood flow and hematocrit. The release of VIP evoked by the mechanical stimulation was calculated as the total amount of VIP discharged in excess of the control release during and after the stimulation period.

Statistical analysis was performed using the Wilcoxon matched-pairs signed-ranks test or the sign test (Siegel, 1956). Differences resulting in P values less than 0·05 were considered significant.

Drugs

Through the cannula in the superior mesenteric artery the following drugs were given: tetrodotoxin (Sigma Chemicals Co., St Louis, Mi.), isopropylnoradrenaline sulphate and 2-bromo-lysergic acid diethylamide (BOL 148, Sandoz). All animals were given atropin (1 mg/kg body wt.) I.V.

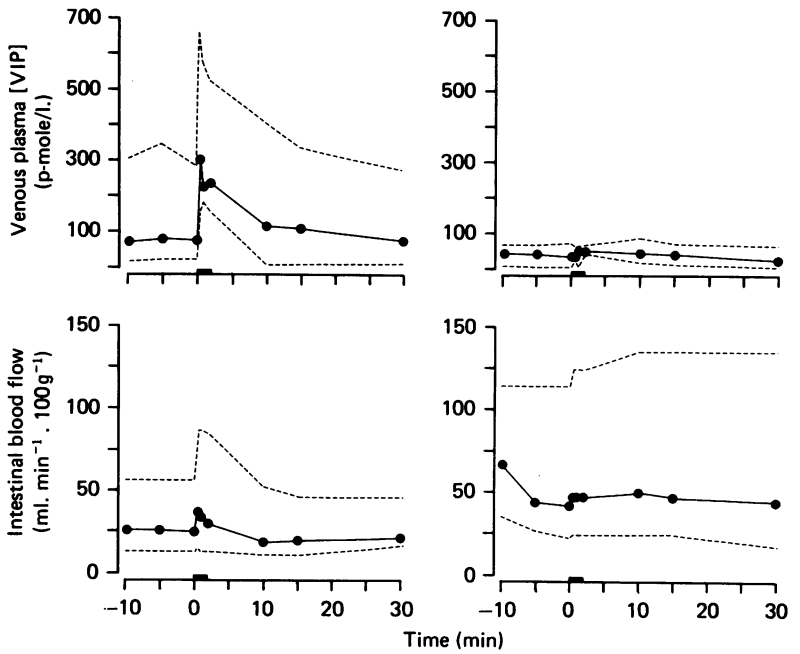

Fig. 2. The effects on venous plasma VIP concentration and intestinal blood flow of mucosal mechanical stimulation for two min (signals) before (left panels) and after (right panels) I.A. administration of tetrodotoxin. Filled circles denote median values and interrupted lines indicate total range (n = 6).

RESULTS

Effects of tetrodotoxin

In five experiments tetrodotoxin (TTX) was given close I.A. in such amounts (about 2 μg/kg body wt.) as to completely abolish the vasoconstriction elicited by electrical stimulation of the regional sympathetic fibres. The intestinal mucosa was then mechanically stimulated for 2 min and intestinal venous samples were taken as indicated in the Methods. Fig. 2 and Table 1 summarize the experimental results before and during TTX blockade.

It is evident from Fig. 2 that the increase of venous VIP concentration produced by mechanical stimulation of the intestinal mucosa was markedly reduced or totally abolished after TTX administration. Concomitantly the vascular response disappeared. Table 1 gives the results from three experiments in which it was possible to determine the release of VIP into blood before and after TTX. It is seen that virtually no VIP was released upon mechanical stimulation after nerve conduction blockade. It was also noted that the 'spontaneous' release of VIP from the gut was decreased after blocking nerve conduction in the intestinal segments.

TABLE 1. Release of VIP during control conditions and in connexion with a 2 min mechanical stimulation before and after close I.A. administration of tetrodotoxin (TTX)

	Rate of VIP release during control (p-mole.min^{-1}.100 g^{-1})		Total release upon mechanical stimulation (p-mole.100 g^{-1})	
Experiment	Before TTX	After TTX	Before TTX	After TTX
1	2·9	1·0	35·1	0·3
2	5·2	1·1	49·4	0
3	1·5	0·5	23·0	0

Effects of 2-bromo-LSD

In five experiments the 5-HT receptor blocking agent 2-bromo-LSD (Gershon, 1977) was administered close I.A. (around 300 μg/kg body wt.) and the effect of mechanical mucosal stimulation on the VIP release and the intestinal hyperaemia were studied. Fig. 3 and Table 2 summarize the results.

Fig. 3 illustrates that the increase of venous plasma VIP concentration seen upon mechanical stimulation was significantly ($P < 0{\cdot}05$) less pronounced after administration of 2-bromo-LSD. It was noted that the decrease of VIP plasma concentration after mechanical stimulation occurred significantly faster after giving the 5-HT blocking agent, the prestimulatory level being reached only 10 min after the mechanical stimulation. Furthermore, in all experiments except one the vascular response seen upon mechanical mucosal stimulation was reduced below 20% of the response seen before giving 2-bromo-LSD.

The results presented in Table 2 show that VIP release upon mechanical stimulation decreased to values below 20% of control after administration of the 5-HT receptor blocking agent in all experiments except one (expt. 5). In the last-mentioned experiment the VIP release was only halved and a marked hyperaemia persisted upon mechanical stimulation, amounting to about 70% of the one seen before drug administration. Table 2 also shows that 'spontaneous' VIP release from the gut during control conditions was reduced in all experiments after treatment with 2-bromo-LSD.

Infusion of 5-HT and isopropylnoradrenaline

Close I.A. infusions of 5-HT (4–18 μg/min) and isopropylnoradrenaline (1–3 μg/min) were performed in five experiments and their effects on the intestinal resistance

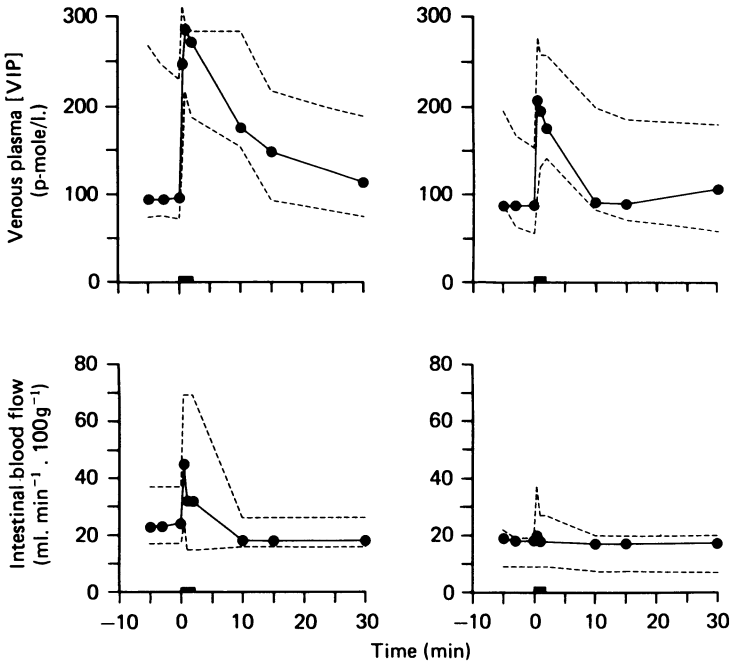

Fig. 3. Venous plasma VIP concentrations and intestinal blood flow observed before, during and after mechanical stimulation of the intestinal mucosa for 2 min (signals). The left panels illustrate the results seen during control conditions while the right ones illustrate the observations made after I.A. injection of 2-bromo-LSD. Filled circles denote median values and interrupted lines indicate total range (n = 5).

TABLE 2. Release of VIP during control conditions and in connexion with a 2 min mechanical stimulation before and after a close I.A. injection of 2-bromo-LSD (BOL)

	Rate of VIP release during control (p-mole.min^{-1}.100 g^{-1})		Total release upon mechanical stimulation (p-mole.100 g^{-1})	
Experiment	Before BOL	After BOL	Before BOL	After BOL
1	2·1	1·4	4·6	0·9
2	1·5	0·7	20·9	2·8
3	2·1	1·0	37·8	3·0
4	0·9	0·8	15·5	2·2
5	0·7	0·6	20·5	10·7

TABLE 3. Release of VIP in connexion with close I.A. infusions of 5-HT and isopropylnoradrenaline. The drugs were infused for 5 min

	Total release (p-mole.100 g^{-1})	
Experiment	5-HT	Isopropylnoradrenaline
1	136	Not determined
2	87	1·0
3	184	9·0
4	209	5·0
5	58	8·6

vessels and VIP release into blood were followed. Fig. 4 and Table 3 summarize the results.

Fig. 4 shows that the venous plasma VIP concentration increased markedly during and after the 5-HT infusion. During the infusion of isopropylnoradrenaline, on the other hand, venous plasma VIP concentration increased only transiently during the first min of infusion and then remained below the preinfusion control level throughout the 30 min observation period. Both drugs infused increased blood flow. Estimating the total VIP release in connexion with the two types of infusions revealed that 5-HT produced a 7–87 times greater release of VIP from the gut than isopropylnoradrenaline (Table 3).

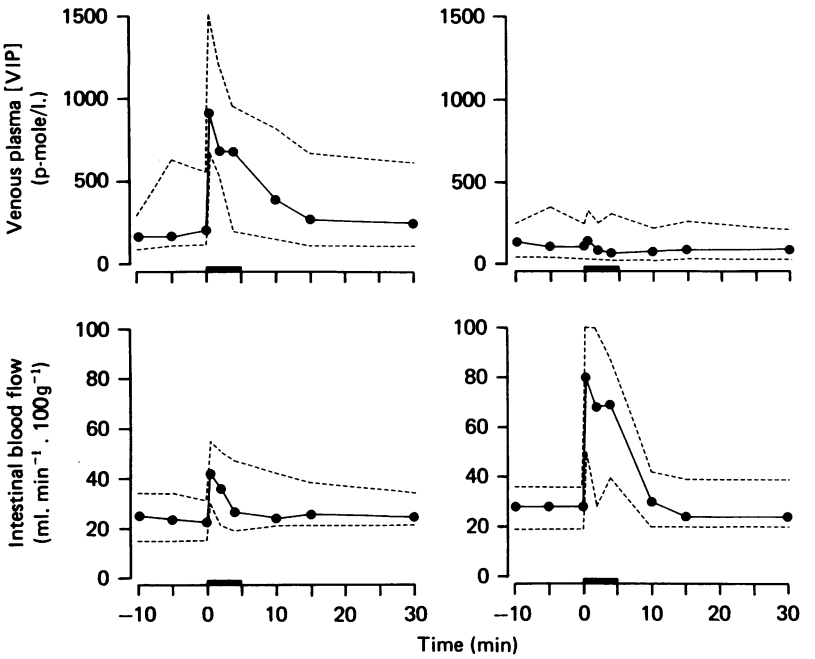

Fig. 4. The effects of close I.A. infusions of 5-HT (left panels) and isopropylnoradrenaline (right panels) on venous plasma VIP concentrations and intestinal blood flow. The infusions were maintained for 5 min (signals). Filled circles denote median values and interrupted lines indicate total range (n = 5).

DISCUSSION

The present study has revealed that the VIP released from the gut upon mechanical stimulation emanates from the VIP-containing neurones since the release is blocked by TTX. This neurogenic release of VIP is apparently markedly decreased by the administration of a 5-HT receptor blocking agent. On the other hand, VIP is released in huge amounts upon close intra-arterial administration of 5-HT. The results are therefore in agreement with the hypothesis outlined in the Introduction and in Fig. 1.

The hypothesis of Fig. 1 implies that 5-HT is not involved in the reflex vasodilatation via a direct effect on the vascular smooth muscle cells or as a neurotransmitter at a synapse but is released from the enterochromaffin cells. This conclusion is based on the following line of evidence. Biber, Fara & Lundgren (1973) showed that it was possible to block with TTX the intestinal vasodilatation induced by close I.A. injection of 5-HT, implying that the hyperaemia was nervously mediated. However, several laboratories have failed to demonstrate 5-HT in the nerveplexa of the mammalian small intestine (Ahlman, Enerbäck, Kewenter & Storm, 1973; Ahlman & Enerbäck, 1974; Dubois & Jacobowitz, 1974; Norberg, 1964; Read & Burnstock, 1968) even in studies using cytofluorometric techniques to differentiate between noradrenaline and serotonin (Ahlman & Enerbäck, 1974). On the other hand, abundant stores of 5-HT were found in the enterochromaffin cells.

The close I.A. infusion of isopropylnoradrenaline also evoked a release of VIP. This effect may be mediated via 5-HT in the way proposed in Fig. 1, since the 5-HT content in the enterochromaffin cells decreases when exposed to isopropylnoradrenaline (Petterson, Dahlström, Larsson, Lundberg, Ahlman & Kewenter, 1978). Quantitatively, the VIP release observed during and after a 5 min infusion of isopropylnoradrenaline was on an average only 20–25 % of that released during and after a 2 min mechanical stimulation period (Tables 1–3). Thus, the VIP releasing effect of isopropylnoradrenaline seems to be fairly weak and the pronounced vasodilatation observed during isopropylnoradrenaline infusion in all probability reflected the direct action of the drug on the vascular smooth muscles.

The results on the haemodynamic effects of TTX and 2-bromo-LSD reported in this study are in full agreement with the observations reported by Biber *et al.* (1971, 1974). These authors provided further experimental evidence for the involvement of 5-HT in the reflex hyperaemia by blocking the vascular effects by making the intestine tachyphylactic towards 5-HT and by the administration of another 5-HT receptor blocking agent, dihydroergotamine. Biber *et al.* (1974) also demonstrated the release of 5-HT from the gut upon mucosal mechanical stimulation. In a recent study by Beubler & Juan (1978) a vasodilatation was observed upon the mechanical stimulation of the intestinal mucosa of the rat. This hyperaemia was not abolished by methysergide, a 5-HT receptor blocking agent. B. Biber (unpublished observations) was also unable to block with methysergide the vasodilatation evoked by close I.A. injection of 5-HT or by mechanical mucosal stimulation. These observations may be ascribed to the fact that methysergide mainly blocks 5-HT receptors on smooth muscles rather than on nervous tissue (Gershon, 1977).

Beubler & Juan (1978) also provide experimental evidence for the release of prostaglandins from the tissue in connexion with the mechanical stimulation of the mucosa. They proposed that this compound was responsible for the vasodilatation although they could only reduce but not abolish the reflex hyperaemia with indomethacin. It cannot, however, be excluded that the prostaglandin release reflected pathophysiological events due to, for example, tissue trauma rather than a truly physiological situation.

The physiological importance of this local nervous reflex is not established but it has been inferred (Biber *et al.* 1971) that the reflex represents one of several mechanisms underlying the functional hyperaemia observed in connexion with digestion.

It seems reasonable to assume that the mechanical stimulation of a food bolus in the gut may produce such a reflex nervous hyperaemia at the site of absorption, by an initial release of 5-HT from the enterochromaffin cells which subsequently activates the VIP-ergic neurones.

The skilful technical assistance of Eva Bengtsson, Pia Larsson, Lisbeth Lindgren, Lene Poulsen, Nina Rasmussen and Anne Sylvest is gratefully acknowledged. The study was supported by grants from the Danish Medical Research Council (J. no. 512-7221), P. Carl Petersens Foundation (B 1048, B 1093), Magnus Bergvalls Foundation, the Swedish Medical Research Council (17X-2855) and the Swedish Society for Medical Sciences.

REFERENCES

AHLMAN, H. & ENERBÄCK, L. (1974). A cytofluorometric study of the myenteric plexus in the guinea-pig. *Cell & Tissue Res.* **153**, 419–434.

AHLMAN, H., ENERBÄCK, L., KEWENTER, J. & STORM, B. (1973). Effects of extrinsic denervation on the fluorescences of monoamines in the small intestine of the cat. *Acta physiol. scand.* **89**, 429–435.

BEUBLER, E. & JUAN, H. (1978). PGE-release, blood flow and transmucosal water movement after mechanical stimulation of the rat jejunal mucosa. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **305**, 91–95.

BIBER, B. (1973). Vasodilator mechanisms in the small intestine. *Acta physiol. scand.* suppl. 401, 1–31.

BIBER, B., FARA, J. & LUNDGREN, O. (1973). Intestinal vascular responses to 5-hydroxytryptamine. *Acta physiol. scand.* **87**, 526–534.

BIBER, B., FARA, J. & LUNDGREN, O. (1974). A pharmacological study of intestinal vasodilator mechanisms in the cat. *Acta physiol. scand.* **90**, 673–683.

BIBER, B., JODAL, M., LUNDGREN, O. & SVANVIK, J. (1970). Intestinal vasodilatation after mechanical stimulation of the jejunal mucosa. *Experientia* **26**, 263–264.

BIBER, B., LUNDGREN, O. & SVANVIK, J. (1971). Studies on the intestinal vasodilatation observed after mechanical stimulation of the mucosa of the gut. *Acta physiol. scand.* **82**, 177–190.

DUBOIS, A. & JACOBOWITZ, D. M. (1974). Failure to demonstrate serotonergic neurons in the myenteric plexus of the rat. *Cell & Tissue Res.* **150**, 493–496.

FAHRENKRUG, J., HAGLUND, U., JODAL, M., LUNDGREN, O., OLBE, L. & SCHAFFALITZKY DE MUCKADELL, O. B. (1978). Nervous release of vasoactive intestinal polypeptide in the gastrointestinal tract of cats: possible physiological implications. *J. Physiol.* **284**, 291–305.

FAHRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. (1977). Radioimmunoassay of vasoactive intestinal polypeptide (VIP) in plasma. *J. Lab. clin. Med.* **89**, 1379–1388.

FAHRENKRUG, J. & SCHAFFALITZKY DE MUCKADELL, O. B. (1978). Distribution of vasoactive intestinal polypeptide (VIP) in the porcine central nervous system. *J. Neurochem.* **31**, 1445–1451.

GERSHON, M. D. (1977). Biochemistry and physiology of serotonergic transmission. In *Handbook of Physiology*, Section 1, The Nervous System, pp. 573–623. Bethesda, Maryland: American Physiological Society.

LARSSON, L.-I., FAHRENKRUG, J., SCHAFFALITZKY DE MUCKADELL, O. B., SUNDLER, F., HÅKANSON, R. & REHFELD, J. F. (1976). Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurones. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3197–3200.

NORBERG, K.-A. (1964). Adrenergic innervation of the intestinal wall studied by fluorescence microscopy. *Int. J. Neuropharmac.* **3**, 379–382.

PETTERSSON, G., DAHLSTRÖM, A., LARSSON, I., LUNDBERG, J. M., AHLMAN, H. & KEWENTER, J. (1978). The release of serotonin from rat duodenal enterochromaffin cells by adrenoceptor agonists studied *in vitro*. *Acta. physiol. scand.* **103**, 219–224.

READ, J. B. & BURNSTOCK, G. (1968). Comparative histochemical studies of adrenergic nerves in the enteric plexuses of vertebrate large intestine. *Comp. Biochem. Physiol.* **27**, 505–517.

SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.